REMARKS

Claims 1, 3-6 and 8-10 were examined. Claim 1 is amended. Claim 13 is added. Claims 1, 3-6, 8-10 and 13 remain in the Application.

I. Claim Rejections – 35 U.S.C. §103

The Patent Office rejects claims 3, 5 and 10 under 35 U.S.C. §103(a) as being obvious over U.S. Patent No. 5,767,153 of Bowman et al. (Bowman) as applied to claims 1, 4, 6, 8 and 9 above, and further in view of U.S. Patent No. 6,342,524 of Hellberg et al. (Hellberg).

The Patent Office cites Bowman for disclosing an opthalmolic emulsion composition that may be administered to the eye in drop form and includes oil, i.e., vegetable and medium chain triglyceride; a water soluble polymer, i.e., polyvinyl pyrrolidone; water and a prostaglandin $F_{2\alpha}$ derivative. Hellberg is cited for disclosing latanaprost in the form of an emulsion containing viscosity building agents such as polyvinyl alcohol. Schneider is cited to show that prostaglandins have low water solubility and can be increased with polyethoxylated caster oil.

Claims 1-10 describe a pharmaceutical composition and an eye drop. Claim 1 provides:

> A pharmaceutical composition comprising an oil-in-water emulsion comprising:
> a prostaglandin $F_{2\alpha}$ derivative, which is at least one member selected from the group consisting of latanoprost, isopropyl unoprostone, travoprost and bimatoprost;
> an oil;
> a water-soluble polymer; and
> water.

Claims 1-9 are not obvious over the cited references, because the cited references do not describe a pharmaceutical composition comprising an oil-in-water emulsion comprising as claimed with a water soluble polymer.

The Patent Office cites Bowman for disclosing a water-soluble polymer. Bowman describes a lightly cross-linked polymer system. Bowman states that "any other suitable lightly cross-linked, water soluble polymer may be employed, for example, polyhydroxyethyl methacrylate or polyvinyl pyrolidone." (Col. 4, lines 6-8.) Polyvinyl pyrrolidone is not cross-

linked. A cross-linked polyvinyl pyrrolidone is crospovidone or polyvinyl polypyrrolidone. See **Exhibit 1** (European Pharmacopoeia) and **Exhibit 2** (The Merck Index). Bowman is directed at using lightly cross-linked water swellable polymers to achieve its formulations. Bowman would presumably not therefore use a non-cross-linked polymer such as polyvinyl pyrrolidone. Bowman teaches "water swellable polymers" which presumably are gels or gel under aqueous conditions. See U.S. Patent No. 5,192,535 (cited at col. 4, lines 1-6 in Bowman as the preferred cross-linked polymers). Bowman's only reference to a possible use of a "water soluble polymer" is at column 4, lines 6-8, where Bowman cites polyhydroxyethylmethacrylate which has a hydrogel form in water. It is true that polyvinyl pyrrolidone is water soluble, but as noted, Applicant believes this polymer is incorrectly identified since it is not cross-linked. Accordingly, Applicant believes reliance on Bowman for teaching the use of water soluble polymers is misplaced.

Hellberg identifies polyvinyl alcohol and polyvinyl pyrrolidones as viscosity building agents. However, there would be no motivation to substitute the cross-linked polymer in Bowman or the viscosity building agent of Hellberg since Bowman is directed at the use of cross-linked polymers. Bowman also points out that prior use of soluble polymers to increase viscosity "can exacerbate the problem [of agglomeration or coalescence] by competing for water, thereby increasing the tendency of the oily disperse phase to separate from the aqueous continuous phase." (Col. 1, lines 27-30.) Accordingly, Bowman teaches against the use of viscosity building agents.

Claims 2-9 depend from claim 1 and contain all of the limitations of that claim. For at least the reasons stated above with respect to claim 1, claims 2-9 are not obvious over the cited references.

Claim 10 describes an eye drop which is an oil in water emulsion comprising latanoprost, medium chain fatty acid triglyceride, polyvinyl alcohol and water. Claim 10 is not obvious over the cited references, because the cited references do not disclose the combination described. With respect to the polyvinyl alcohol component, this component is not a cross-linked polymer. Further, although Hellberg describes polyvinyl alcohol as a suitable viscosity builder, combining

or substituting polyvinyl alcohol where the cross-linked polymers in Bowman is not taught, suggested or predicted since Bowman teaches away from such usage.

For the above-stated reasons, Applicant respectfully requests that the Patent Office withdraw the rejection to claims 1, 3-6 and 8-10 under 35 U.S.C. §103(a).

II. Unexpected Result of PG Compounds Other Than "Latanoprost"

Applicant asserted in its response filed January 31, 2011 that an emulsion of latanoprost showed excellent stability of the drug in an aqueous composition as shown in Table 2 of the Application and that a person of skill in the art can expect that other prostaglandin derivatives recited in claim 1 of the Application would be stabilized by emulsifying a formulation according to the latanoprost composition.

The Patent Office countered the unexpected result argument by noting that Schneider teaches that oils increase the stability of prostaglandins and the concentration of the oil is a "result effective" variable. Schneider teaches the use of polycthoxylated caster oil. Applicant believes polyethoxylated caster oil is "an oil" in name only. Polyethoxylated caster oil is a non-ionic surfactant, not an oil, such as described in the Application (e.g., easter oil). See **Exhibit 3** (Phase 1 and Pharmacokinetic Study of ABI-007, a Cremophor-free, Protein-stabilized, Nanoparticle Formulation of Paclitaxel) and **Exhibit 4** (BASF Technical Leaflet for Cremophor® EL).

III. New Claim 13

Applicant adds new claim 13 directed at water soluble polymers identified in the Application at, page 6, line 22 through page 7, line 4. Applicant believes claim 13 is distinguishable from the cited art references for at least the reasons stated above with respect to claims 1-10.

CONCLUSION

In view of the foregoing, it is believed that all claims now pending patentably define the subject invention over the prior art of record and are in condition for allowance and such action is earnestly solicited at the earliest possible date.

If necessary, the Commissioner is hereby authorized in this, concurrent and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2666 for any additional fees required under 37 C.F.R. §§ 1.16 or 1.17, particularly extension of time fees.

Respectfully submitted,

BLAKELY, SOKOLOFF, TAYLOR, & ZAFMAN LLP

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CERTIFICATE OF TRANSMISSION

I hereby certify that this correspondence is being submitted electronically via EFS Web on the date shown below to the United States Patent and Trademark Office.

Nedy Calderon 7/28/11
Nedy Calderon Date

EUROPEAN PHARMACOPOEIA

SIXTH EDITION

Volume 2

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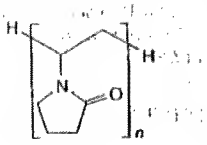
EXHIBIT 1

01/2008:0892
corrected 6.0

CROSPOVIDONE

Crospovidonum



$(C_6H_9NO)_n$ M_r $(111.1)_n$
[9003-39-8]

DEFINITION

Crospovidone is a cross-linked homopolymer of 1-ethenylpyrrolidin-2-one. It is available in different degrees of powder fineness (type A and type B).

Content: 11.0 per cent to 12.8 per cent of nitrogen (N; A_r 14.01) (dried substance).

CHARACTERS

Appearance: white or yellowish-white powder or flakes, hygroscopic.

Solubility: practically insoluble in water, in alcohol and in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *Ph. Eur. reference spectrum of crospovidone.*

B. Suspend 1 g in 10 ml of *water R*, add 0.1 ml of *0.05 M iodine* and shake for 30 s. Add 1 ml of *starch solution R* and shake. No blue colour develops within 30 s.

C. To 10 ml of *water R*, add 0.1 g and shake. A suspension is formed and no clear solution is obtained within 15 min.

D. Weigh a suitable quantity of the substance to be examined (for example 10 mg to 100 mg) and suspend it in 10.0 ml of *water R*, adding a wetting agent. Observe under a microscope at a suitable magnification using a calibrated ocular micrometer. If the majority of particles are in the range 50 µm to 300 µm, the product is classified as type A. If almost all the particles are below 50 µm, the product is classified as type B.

TESTS

Peroxides. Type A: maximum 400 ppm expressed as H_2O_2; type B: maximum 1000 ppm expressed as H_2O_2.

Suspend 2.0 g in 50 ml of *water R*. To 25 ml of this suspension add 2 ml of *titanium trichloride-sulphuric acid reagent R*. Allow to stand for 30 min and filter. The absorbance (*2.2.25*) of the filtrate, measured at 405 nm using a mixture of 25 ml of a filtered 40 g/l suspension of the substance to be examined and 2 ml of a 13 per cent *V/V* solution of *sulphuric acid R* as the compensation liquid has a maximum of 0.35.

For type B use 10 ml of the suspension diluted to 25 ml with *water R* for the test.

Water-soluble substances: maximum 1.0 per cent.

Transfer 25.0 g to a 400 ml beaker, add 200 ml of *water R* and stir for 1 h using a magnetic stirrer. Transfer the suspension to a 250.0 ml volumetric flask, rinsing with *water R*, and dilute to volume with the same solvent. Allow the bulk of the solids to settle. Filter about 100 ml of the almost clear supernatant liquid through a 0.45 µm membrane filter, protected by superimposing a 3 µm membrane filter. While filtering, stir the liquid above the filter manually or by means of a mechanical stirrer, taking care not to damage the filter. Transfer 50.0 ml of the clear filtrate to a tared 100 ml beaker, evaporate to dryness and dry at 105-110 °C for 3 h. The residue weighs a maximum of 50 mg.

Impurity A. Liquid chromatography (*2.2.29*).

Test solution. Suspend 1.250 g in 50.0 ml of *methanol R* and shake for 60 min. Leave the bulk to settle and filter through a 0.2 µm filter.

Reference solution (a). Dissolve 50 mg of *1-vinylpyrrolidin-2-one R* in *methanol R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of the solution to 100.0 ml with *methanol R*. Dilute 5.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (b). Dissolve 10 mg of *1-vinylpyrrolidin-2-one R* and 0.50 g of *vinyl acetate R* in *methanol R* and dilute to 100 ml with the same solvent. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase.

Precolumn:
- *size*: l = 0.025 m, Ø = 4 mm,
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Column:
- *size*: l = 0.25 m, Ø = 4 mm,
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm),
- *temperature*: 40 °C.

Mobile phase: *acetonitrile R, water R* (10:90 *V/V*).

Flow rate: adjusted so that the retention time of the peak corresponding to impurity A is about 10 min.

Detection: spectrophotometer at 235 nm.

Injection: 50 µl. After each injection of the test solution, wash the precolumn by passing the mobile phase backward, at the same flow rate as applied in the test, for 30 min.

System suitability:
- *resolution*: minimum of 2.0 between the peaks corresponding to impurity A and to vinyl acetate in the chromatogram obtained with reference solution (b),
- *repeatability*: maximum relative standard deviation of 2.0 per cent after 5 injections of reference solution (a).

Limits:
- *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (10 ppm).

Heavy metals (*2.4.8*): maximum 10 ppm.

2.0 g complies with limit test D. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying (*2.2.32*): maximum 5.0 per cent, determined on 0.500 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Place 100.0 mg of the substance to be examined (m mg) in a combustion flask, add 5 g of a mixture of 1 g of *copper sulphate R*, 1 g of *titanium dioxide R* and 33 g of *dipotassium sulphate R*, and 3 glass beads. Wash any adhering particles from the neck into the flask with a small quantity of *water R*. Add 7 ml of *sulphuric acid R*, allowing it to run down the sides of the flask, and mix the contents by rotation. Close the mouth of the flask loosely, for example by means of a glass bulb with a short stem, to

avoid excessive loss of sulphuric acid. Heat gradually at first, then increase the temperature until there is vigorous boiling with condensation of sulphuric acid in the neck of the flask; precautions are to be taken to prevent the upper part of the flask from becoming overheated. Continue the heating for 45 min. Cool, dissolve the solid material by cautiously adding to the mixture 20 ml of *water R*, cool again and place in a steam-distillation apparatus. Add 30 ml of *strong sodium hydroxide solution R* through the funnel, rinse the funnel cautiously with 10 ml of *water R* and distil immediately by passing steam through the mixture. Collect 80-100 ml of distillate in a mixture of 30 ml of a 40 g/l solution of *boric acid R* and 0.05 ml of *bromocresol green-methyl red solution R* and enough *water R* to cover the tip of the condenser. Towards the end of the distillation lower the receiver so that the tip of the condenser is above the surface of the acid solution and rinse the end part of the condenser with a small quantity of *water R*. Titrate the distillate with *0.025 M sulphuric acid* until the colour of the solution changes from green through pale greyish-blue to pale greyish-red-purple (n_1 ml of *0.025 M sulphuric acid*).

Repeat the test using about 100 mg of *glucose R* in place of the substance to be examined (n_2 ml of *0.025 M sulphuric acid*).

$$\text{Percentage content of nitrogen} = \frac{0.7004\,(n_1 - n_2)}{m} \times 100$$

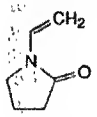
STORAGE

In an airtight container.

LABELLING

The label states the type (type A or type B).

IMPURITIES



A. 1-ethenylpyrrolidin-2-one (1-vinylpyrrolidin-2-one).

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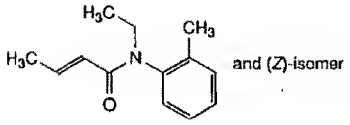
CROTAMITON

Crotamitonum



and (Z)-isomer

$C_{13}H_{17}NO$ M_r 203.3

[483-63-6]

DEFINITION

N-Ethyl-*N*-(2-methylphenyl)but-2-enamide.

Content:

- *sum of the E- and Z-isomers*: 96.0 per cent to 102.0 per cent;
- *Z-isomer*: maximum 15.0 per cent.

CHARACTERS

Appearance: colourless or pale yellow, oily liquid.

Solubility: slightly soluble in water, miscible with ethanol (96 per cent).

At low temperatures it may partly or completely solidify.

IDENTIFICATION

First identification: B.

Second identification: A, C, D.

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution. Dissolve 25.0 mg in *cyclohexane R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of this solution to 10.0 ml with *cyclohexane R*.

Spectral range: 220-300 nm.

Absorption maximum: at 242 nm.

Specific absorbance at the absorption maximum: 300 to 330.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *crotamiton CRS*.

C. Thin-layer chromatography (*2.2.27*).

Test solution. Dissolve 25 mg of the substance to be examined in *anhydrous ethanol R* and dilute to 10 ml with the same solvent.

Reference solution. Dissolve 25 mg of *crotamiton CRS* in *anhydrous ethanol R* and dilute to 10 ml with the same solvent.

Plate: *TLC silica gel F_{254} plate R*.

Mobile phase: shake 98 volumes of *methylene chloride R* with 2 volumes of *concentrated ammonia R*, dry over *anhydrous sodium sulphate R*, filter and mix 97 volumes of the filtrate with 3 volumes of *2-propanol R*.

Application: 5 µl.

Development: over a path of 15 cm.

Drying: in air.

Detection: examine in ultraviolet light at 254 nm.

Results: the principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

D. To 10 ml of a saturated solution add a few drops of a 3 g/l solution of *potassium permanganate R*. A brown colour is produced and a brown precipitate is formed on standing.

TESTS

Relative density (*2.2.5*): 1.006 to 1.011.

Refractive index (*2.2.6*): 1.540 to 1.542.

Free amines: maximum 500 ppm, expressed as ethylaminotoluene.

Dissolve 5.00 g in 16 ml of *methylene chloride R* and add 4.0 ml of *glacial acetic acid R*. Add 0.1 ml of *metanil yellow solution R* and 1.0 ml of *0.02 M perchloric acid*. The solution is red-violet.

Chlorides: maximum 100 ppm.

Boil 5.0 g under a reflux condenser for 1 h with 25 ml of *ethanol (96 per cent) R* and 5 ml of a 200 g/l solution of *sodium hydroxide R*. Cool, add 5 ml of *water R* and shake with 25 ml of *ether R*. Dilute the lower layer to 20 ml with *water R*; add 5 ml of *nitric acid R*, dilute to 50 ml with *water R* and add 1 ml of a freshly prepared 50 g/l solution of *silver nitrate R*. Any opalescence in the solution is not more intense than that in a mixture of 1 ml of a freshly prepared 50 g/l solution of *silver nitrate R* and a solution prepared by

TESTS

pH (*2.2.3*): 5.0 to 8.0.

Shake 5.0 g with 25.0 ml of *carbon dioxide-free water R* for 60 s. Allow to stand for 15 min.

Foreign matter. Examined under a microscope using a mixture of equal volumes of *glycerol R* and *water R*, not more than traces of matter other than starch granules are present. No starch grains of any other origin are present.

Oxidising substances (*2.5.30*): maximum 20 ppm, calculated as H_2O_2.

Sulphur dioxide (*2.5.29*): maximum 50 ppm.

Iron (*2.4.9*): maximum 10 ppm.

Shake 1.5 g with 15 ml of *dilute hydrochloric acid R*. Filter. The filtrate complies with the limit test for iron.

Loss on drying (*2.2.32*): maximum 20.0 per cent, determined on 1.000 g by drying in an oven at 130 °C for 90 min.

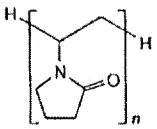
Sulphated ash (*2.4.14*): maximum 0.6 per cent, determined on 1.0 g.

Microbial contamination. Total viable aerobic count (*2.6.12*) not more than 10^3 bacteria and not more than 10^2 fungi per gram, determined by plate count. It complies with the test for *Escherichia coli* (*2.6.13*).

01/2008:0685

POVIDONE

Povidonum



$C_{6n}H_{9n+2}N_nO_n$

[9003-39-8]

DEFINITION

α-Hydro-ω-hydropoly[1-(2-oxopyrrolidin-1-yl)ethylene]. It consists of linear polymers of 1-ethenylpyrrolidin-2-one.

Content: 11.5 per cent to 12.8 per cent of nitrogen (N; A_r 14.01) (anhydrous substance).

The different types of povidone are characterised by their viscosity in solution, expressed as a *K*-value.

The *K*-value of povidone having a stated *K*-value of 15 or less is 85.0 per cent to 115.0 per cent of the stated value.

The *K*-value of povidone having a stated *K*-value or a stated *K*-value range with an average of more than 15 is 90.0 per cent to 108.0 per cent of the stated value or of the average of the stated range.

CHARACTERS

Appearance: white or yellowish-white; hygroscopic powder or flakes.

Solubility: freely soluble in water, in ethanol (96 per cent) and in methanol, very slightly soluble in acetone.

IDENTIFICATION

First identification: *A*, *E*.

Second identification: *B*, *C*, *D*, *E*.

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation: dry the substances beforehand at 105 °C for 6 h. Record the spectra using 4 mg of substance.

Comparison: *povidone CRS*.

B. To 0.4 ml of solution S1 (see Tests) add 10 ml of *water R*, 5 ml of *dilute hydrochloric acid R* and 2 ml of *potassium dichromate solution R*. An orange-yellow precipitate is formed.

C. To 1 ml of solution S1 add 0.2 ml of *dimethylaminobenzaldehyde solution R1* and 0.1 ml of *sulphuric acid R*. A pink colour is produced.

D. To 0.1 ml of solution S1 add 5 ml of *water R* and 0.2 ml of *0.05 M iodine*. A red colour is produced.

E. It is freely soluble in *water R*.

TESTS

Solution S. Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent. Add the substance to be examined to the water in small portions with magnetic stirring.

Solution S1. Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 25 ml with the same solvent. Add the substance to be examined to the water in small portions with magnetic stirring.

Appearance of solution. Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution B_6, BY_6 or R_6 (*2.2.2, Method II*).

pH (*2.2.3*): 3.0 to 5.0 for solution S, for povidone having a stated *K*-value of not more than 30; 4.0 to 7.0 for solution S, for povidone having a stated *K*-value of more than 30.

Viscosity, expressed as *K*-value. For povidone having a stated value of 18 or less, use a 50 g/l solution. For povidone having a stated value of more than 18, use a 10 g/l solution. For povidone having a stated value of more than 95, use a 1.0 g/l solution. Allow to stand for 1 h and determine the viscosity (*2.2.9*) of the solution at 25 °C, using viscometer No.1 with a minimum flow time of 100 s. Calculate the *K*-value using the following expression:

$$\frac{1.5\log\eta - 1}{0.15 + 0.003c} + \frac{\sqrt{300c\log\eta + (c + 1.5c\log\eta)^2}}{0.15c + 0.003c^2}$$

c = concentration of the substance to be examined, calculated with reference to the anhydrous substance, in grams per 100 ml;

η = viscosity of the solution relative to that of *water R*.

Aldehydes: maximum 500 ppm, expressed as acetaldehyde.

Test solution. Dissolve 1.0 g of the substance to be examined in *phosphate buffer solution pH 9.0 R* and dilute to 100.0 ml with the same solvent. Stopper the flask and heat at 60 °C for 1 h. Allow to cool.

Reference solution. Dissolve 0.140 g of *acetaldehyde ammonia trimer trihydrate R* in *water R* and dilute to 200.0 ml with the same solvent. Dilute 1.0 ml of this solution to 100.0 ml with *phosphate buffer solution pH 9.0 R*.

Into 3 identical spectrophotometric cells with a path length of 1 cm, introduce separately 0.5 ml of the test solution, 0.5 ml of the reference solution and 0.5 ml of *water R* (blank). To each cell, add 2.5 ml of *phosphate buffer solution pH 9.0 R* and 0.2 ml of *nicotinamide-adenine dinucleotide solution R*. Mix and stopper tightly. Allow to stand at 22 ± 2 °C for 2-3 min and measure the absorbance (*2.2.25*) of each solution at 340 nm, using *water R* as the compensation liquid. To each cell, add 0.05 ml of *aldehyde*

dehydrogenase solution R, mix and stopper tightly. Allow to stand at 22 ± 2 °C for 5 min. Measure the absorbance of each solution at 340 nm using *water R* as the compensation liquid.

Calculate the content of aldehydes using the following expression:

$$\frac{(A_{t2} - A_{t1}) - (A_{b2} - A_{b1})}{(A_{s2} - A_{s1}) - (A_{b2} - A_{b1})} \times \frac{100\,000 \times C}{m}$$

A_{t1} = absorbance of the test solution before the addition of aldehyde dehydrogenase;

A_{t2} = absorbance of the test solution after the addition of aldehyde dehydrogenase;

A_{s1} = absorbance of the reference solution before the addition of aldehyde dehydrogenase;

A_{s2} = absorbance of the reference solution after the addition of aldehyde dehydrogenase;

A_{b1} = absorbance of the blank before the addition of aldehyde dehydrogenase;

A_{b2} = absorbance of the blank after the addition of aldehyde dehydrogenase;

m = mass of povidone calculated with reference to the anhydrous substance, in grams;

C = concentration of acetaldehyde in the reference solution, calculated from the weight of the acetaldehyde ammonia trimer trihydrate with the factor 0.72, in milligrams per millilitre.

Peroxides: maximum 400 ppm, expressed as H_2O_2.

Dissolve 2.0 g in 50 ml of *water R*. To 25 ml of this solution, add 2 ml of *titanium trichloride-sulphuric acid reagent R*. Allow to stand for 30 min. The absorbance (*2.2.25*) of the solution, measured at 405 nm using a mixture of 25 ml of a 40 g/l solution of the substance to be examined and 2 ml of a 13 per cent *V/V* solution of *sulphuric acid R* as the compensation liquid, is not greater than 0.35.

Hydrazine. Thin-layer chromatography (*2.2.27*). *Use freshly prepared solutions.*

Test solution. Dissolve 2.5 g of the substance to be examined in 25 ml of *water R*. Add 0.5 ml of a 50 g/l solution of *salicylaldehyde R* in *methanol R*, mix and heat in a water-bath at 60 °C for 15 min. Allow to cool, add 2.0 ml of *toluene R*, shake for 2 min and centrifuge. Use the clear supernatant layer.

Reference solution. Dissolve 9 mg of *salicylaldehyde azine R* in *toluene R* and dilute to 100 ml with the same solvent. Dilute 1 ml of the solution to 10 ml with *toluene R*.

Plate: *TLC silanised silica gel plate R*.

Mobile phase: *water R, methanol R* (1:2 *V/V*).

Application: 10 µl.

Development: over a path of 15 cm.

Drying: in air.

Detection: examine in ultraviolet light at 365 nm.

Limit:

– *hydrazine*: any spot corresponding to salicylaldehyde azine in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution (1 ppm).

Impurity A. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 0.25 g of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a). Dissolve 50 mg of *1-vinylpyrrolidin-2-one R* in *methanol R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of the solution to 100.0 ml with *methanol R*. Dilute 5.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (b). Dissolve 10 mg of *1-vinylpyrrolidin-2-one R* and 0.5 g of *vinyl acetate R* in *methanol R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase.

Precolumn:

– *size*: *l* = 0.025 m, Ø = 4 mm;

– *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Column:

– *size*: *l* = 0.25 m, Ø = 4 mm;

– *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);

– *temperature*: 40 °C.

Mobile phase: *acetonitrile R, water R* (10.90 *V/V*).

Flow rate: adjusted so that the retention time of the peak corresponding to impurity A is about 10 min.

Detection: spectrophotometer at 235 nm.

Injection: 50 µl. After injection of the test solution, wait for about 2 min and wash the precolumn by passing the mobile phase backwards, at the same flow rate applied in the test, for 30 min.

System suitability:

– *resolution*: minimum 2.0 between the peaks due to impurity A and to vinyl acetate in the chromatogram obtained with reference solution (b);

– *repeatability*: maximum relative standard deviation of 2.0 per cent after 5 injections of reference solution (a).

Limit:

– *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (10 ppm).

Impurity B. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 100 mg of the substance to be examined in *water R* and dilute to 50.0 ml with the same solvent.

Reference solution. Dissolve 100 mg of *2-pyrrolidone R* in *water R* and dilute to 100 ml with the same solvent. Dilute 3.0 ml to 50.0 ml with *water R*.

Precolumn:

– *size*: *l* = 0.025 m, Ø = 4 mm;

– *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Column:

– *size*: *l* = 0.25 m, Ø = 4 mm;

– *stationary phase*: spherical *aminohexadecylsilyl silica gel for chromatography R* (5 µm);

– *temperature*: 30 °C.

Mobile phase: *water R*, adjusted to pH 2.4 with *phosphoric acid R*.

Flow rate: 1 ml/min.

Detection: spectrophotometer at 205 nm. A detector is placed between the precolumn and the analytical column. A 2nd detector is placed after the analytical column.

Injection: 10 µl. When impurity B has left the precolumn (after about 1.2 min) switch the flow directly from the pump to the analytical column. Before the next chromatogram is run, wash the precolumn by reversed flow.

System suitability:

– *symmetry factor*: maximum 2.0 for the peak due to impurity B.

Limit:

– *impurity B*: not more than the area of the principal peak in the chromatogram obtained with the reference solution (3.0 per cent).

Heavy metals (*2.4.8*): maximum 10 ppm.

2.0 g complies with test D. Prepare the reference solution using 2.0 ml of *lead standard solution (10 ppm Pb) R*.

Water (*2.5.12*): maximum 5.0 per cent, determined on 0.500 g.

Sulphated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Place 100.0 mg of the substance to be examined (*m* mg) in a combustion flask, add 5 g of a mixture of 1 g of *copper sulphate R*, 1 g of *titanium dioxide R* and 33 g of *dipotassium sulphate R*, and 3 glass beads. Wash any adhering particles from the neck into the flask with a small quantity of *water R*. Add 7 ml of *sulphuric acid R*, allowing it to run down the insides of the flask, and mix the contents by rotation. Close the mouth of the flask loosely, for example by means of a glass bulb with a short stem, to avoid excessive loss of sulphuric acid. Heat gradually at first, then increase the temperature until there is vigorous boiling with condensation of sulphuric acid in the neck of the flask; precautions are to be taken to prevent the upper part of the flask from becoming overheated. Continue the heating for 45 min. Cool, dissolve the solid material by cautiously adding to the mixture 70 ml of *water R*, cool again and place in a steam-distillation apparatus. Add 30 ml of *strong sodium hydroxide solution R* through the funnel, cautiously rinse the funnel with 10 ml of *water R* and distil immediately by passing steam through the mixture. Collect about 80-100 ml of distillate in a mixture of 30 ml of a 40 g/l solution of *boric acid R* and 3 drops of *bromocresol green-methyl red solution R* and enough *water R* to cover the tip of the condenser. Towards the end of the distillation, lower the receiver so that the tip of the condenser is above the surface of the acid solution and rinse the end part of the condenser with a small quantity of *water R*. Titrate the distillate with *0.025 M sulphuric acid* until the colour of the solution changes from green through pale greyish-blue to pale greyish-red-purple (n_1 ml of *0.025 M sulphuric acid*).

Repeat the test using about 100.0 mg of *glucose R* in place of the substance to be examined (n_2 ml of *0.025 M sulphuric acid*).

Calculate the percentage content of nitrogen using the following expression:

$$\frac{0.7004\ (n_1 - n_2)}{m} \times 100$$

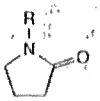
STORAGE

In an airtight container.

LABELLING

The label indicates the nominal *K*-value.

IMPURITIES



A. R = $CH{=}CH_2$: 1-ethenylpyrrolidin-2-one (1-vinylpyrrolidin-2-one),

B. R = H: pyrrolidin-2-one (2-pyrrolidone).

01/2008:1142
corrected 6.0

POVIDONE, IODINATED

Povidonum iodinatum

DEFINITION

Complex of iodine and povidone.

Content: 9.0 per cent to 12.0 per cent of available iodine (dried substance).

PRODUCTION

It is produced using povidone that complies with the monograph on *Povidone (0685)*, except that the povidone used may contain not more than 2.0 per cent of formic acid and not more than 8.0 per cent of water.

CHARACTERS

Appearance: yellowish-brown or reddish-brown, amorphous powder.

Solubility: soluble in water and in ethanol (96 per cent), practically insoluble in acetone.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *iodinated povidone CRS*.

B. Dissolve 10 mg in 10 ml of *water R* and add 1 ml of *starch solution R*. An intense blue colour is produced.

C. Dissolve 0.1 g in 5 ml of *water R* and add a 10 g/l solution of *sodium sulphite R* dropwise, until the solution becomes colourless. Add 2 ml of *potassium dichromate solution R* and 1 ml of *hydrochloric acid R*. A light brown precipitate is formed.

TESTS

pH (*2.2.3*): 1.5 to 5.0.

Dissolve 1.0 g in 10 ml of *carbon dioxide-free water R*.

Iodide: maximum 6.0 per cent (dried substance).

Dissolve 0.500 g in 100 ml of *water R*. Add *sodium metabisulphite R* until the colour of the iodine has disappeared. Add 25.0 ml of *0.1 M silver nitrate*, 10 ml of *nitric acid R* and 5 ml of *ferric ammonium sulphate solution R2*. Titrate with *0.1 M ammonium thiocyanate*. Carry out a blank titration.

1 ml of *0.1 M silver nitrate* is equivalent to 12.69 mg of total iodine. From the percentage of total iodine, calculated with reference to the dried substance, subtract the percentage of available iodine as determined in the assay to obtain the percentage of iodide.

Loss on drying (*2.2.32*): maximum 8.0 per cent, determined on 0.500 g by drying in an oven at 105 °C for 3 h.

Sulphated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

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ing alcohol. When dissolved in its own wt of water, the temp drops about 30°. The aq soln is neutral. *Keep well closed.* LD_{50} orally in mice, rats: 594, 854 mg/kg, Andersen, Chen, *J. Am. Pharm. Assoc.* **29,** 152 (1940).

Caution: May cause skin eruptions, psychosis.

USE: Manuf artificial mustard oil; printing and dyeing textiles; in photography as intensifier; in analytical chemistry. The sodium salt now is replacing it for most of these uses.

THERAPEUTIC: Hypotensive.

7777. Potassium Thiosulfate. [10294-66-3] Potassium hyposulfite. $K_2O_3S_2$; mol wt 190.32. K 41.09%, O 25.22%, S 33.70%. $K_2S_2O_3$

Colorless, hygroscopic crystals. Sol in water. Insol in alcohol. May crystallize with 0.33 to 1.5 mol H_2O. *Keep well closed.*

7778. Potassium Titanyl Oxalate. [14402-67-6] Dipotassium bis[ethanedioato(2-)-*O,O'*]oxotitanate(2-); titanium potassium oxalate; titanyl potassium oxalate. $C_4K_2O_9Ti$; mol wt 318.10. C 15.10%, K 24.58%, O 45.27%, Ti 15.05%. $K_2TiO(C_2O_4)_2$.

Crystals or cryst powder. Very sol in water.

Dihydrate. Potassium oxodioxalatodiaquotitanate(IV)

USE: As mordant in dyeing cotton and leather.

7779. Potassium Triiodomercurate(II) Solution. [22330-18-3] Mercuric potassium iodide soln; potassium mercuric iodide soln; soln potassium iodohydrargyrate; Channing's soln; Thoulet's soln. Prepd by dissolving 1 g HgI_2 and 0.8 g KI in water to make 100 ml. *Poison!*

USE: As reagent for alkaloids.

THERAPEUTIC: Antiseptic (topical); disinfectant.

7780. Potassium Tungstate(VI). [7790-60-5] K_2O_4W; mol wt 326.04. K 23.98%, O 19.63%, W 56.39%. K_2WO_4. Crystallizes also with 2H₂O.

Heavy, deliquesc, cryst powder. d 3.127; mp 921°. Sol in about 2 parts cold, about 0.7 part boiling water. Insol in alcohol. *Keep well closed.*

7781. Potassium Uranate(VI). [7790-63-8] Potassium diuranate, mannum oxide orange. $K_2O_7U_2$; mol wt 666.25. K 11.74%, O 16.81%, U 71.45%. $K_2U_2O_7$.

Orange powder. Insol in water; sol in acids.

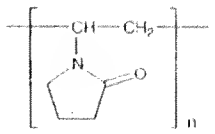
USE: Painting on porcelain.

7782. Potassium Xanthogenate. [140-89-6] Carbonodithioic acid *O*-ethyl ester potassium salt; ethylxanthic acid potassium salt; potassium ethyldithiocarbonate; potassium ethyl xanthogenate; potassium ethylxanthate. $C_3H_5KOS_2$; mol wt 160.30. C 22.48%, H 3.14%, K 24.39%, O 9.98%, S 40.01%. $C_2H_5OCS_2K$. Made by treating an alcoholic soln of CS_2 with alcoholic KOH. Usually contains 8.10% H₂O.

White to pale yellow crystals or cryst powder. Very sol in water; sol in alcohol. The aq soln is strongly alkaline. *Keep well closed and protected from light.*

USE: As reagent in analytical chemistry.

7783. Povidone. [9003-39-8] 1-Ethenyl-2-pyrrolidinone homopolymer; 1-vinyl-2-pyrrolidinone polymers; poly[1-(2-oxo-1-pyrrolidinyl)ethylene]; polyvinylpyrrolidone; polyvidone; PVP; Kollidon; Luviskol; Periston; Plasdone; Protagent. Homopolymer of *N*-vinyl-2-pyrrolidone, produced commercially as a series of products having mean mol wts ranging from 2,500 to 1,000,000. Prepd by free radical polymerization of the monomer. *See* J. W. Reppe, *Acetylene Chemistry,* (PB Report 18852 s, U.S. Dept. Commerce, 1949) pp 68-72. Review of clinical use and early literature: W. Wessel *et al., Arzneimittel-Forsch.* **21,** 1468-1482 (1971); of synthesis and physical properties: H. Watson, *Polymers Paint Colour J.* **161,** 637-614 (1972); P. Haal *et al., Polymer J.* **17,** 113-152 (1985); of use in cosmetics: F. G. M. Vogel, *Soap Cosmet. Chem. Special.* **65,** 42-47, 128 (1989). Book: *PVP: A Critical Review of the Kinetics and Toxicology of Polyvinylpyrrolidone (Povidone),* B. V. Robinson *et al.*, Eds. (Lewis Publishers, Chelsea, MI, 1990) 209 pp. Comprehensive description: C. M. Adeyeye, P. Barabas, *Anal. Profiles Drug Subs. Excip.* **22,** 585-685 (1993). Size exclusion chromatography: C. Wu *et al., Chromatog. Sci. Ser.* **69,** 311 (1995).



White, hygroscopic powder. Sol in water, alcohol, chloroform, formic acid, acetic acid, *N*-methylpyrrolidinone, methylcyclohexanone, dichloromethane, ethylenediamine, glycerol, diethyleneglycol, PEG-400. Insol in xylene, toluene, diethyl ether, ethylacetate, acetone, cyclohexanone, chlorobenzene, dioxane, carbon tetrachloride, mineral oil.

Crospovidone. Polyvinylpolypyrrolidone; PVPP; Divergan, Kollidon CL, Polyclar; Polyplasdone XL. Crosslinked insoluble homopolymer of NVP. Review of properties and applications: A. H. Bronnsack in *Proc. Intl. Symp. Povidone,* G. A. Digenis, J. Ansell, Eds. (Univ. Kentucky, Coll. Pharmacy, Lexington, 1983) pp 471-490. Comprehensive description: E. S. Barabas, C. M. Adeyeye, *Anal. Profiles Drug Subs. Excip.* **24,** 87-163 (1996). Free flowing, white, almost tasteless powder. Hygroscopic; swells on contact with water. Insol in water, strong mineral acids, caustic solns, and common organic solvents.

Monomer. [88-12-0] 1-Ethenyl-2-pyrrolidinone, *N*-vinyl-2-pyrrolidinone, NVP. C_6H_9NO; mol wt 111.14. Clear to light straw colored liquid; bp_{14} 96°, bp_{760} 193°. Freezing pt 13.5°; d_4^{25} 1.04; n_D^{25} 1.511. Flash pt (open cup) 100.5°C (213°F). Viscosity (25°): 2.07 cps. Sol in water and many organic solvents.

Complex with iodine, *see* Povidone-Iodine.

USE: Povidone as pharmaceutic aid (dispersing, suspending and viscosity-increasing agent; tablet coating and binder). Thickener, dispersant, lubricant; film forming agent and binder in cosmetics. Stabilizer, diluent, and dye dispersant in food. Dye dispersant in paper and textiles. Adhesive, paper coating, coating and processing aid in photographic products. Manuf of plastics and rubber. Cryoprotectant for biological samples. Crospovidone as pharmaceutic aid (tablet binder and disintegrant); clarifying and stabilizing agent in beverages. Monomer as dispersant and wetting agent in pigments.

THERAPEUTIC: Povidone formerly as a synthetic blood plasma expander. Crospovidone as antidiarrheal.

7784. Povidone-Iodine. [25655-41-8] 1-Ethenyl-2-pyrrolidinone homopolymer compd with iodine; 1-vinyl-2-pyrrolidinone polymers, iodine complex; iodine polyvinylpyrrolidone complex; polyvinylpyrrolidone iodine complex; PVP-I; Betadine; Betasodona; Braunol; Braunosan II; Disadine DP; Efodine; Inadine; Isodine; Pitvoiodine; Traumasept. An iodophor, *q.v.*; prepd by Beller, Hosmer, **US 2706701**, Hosmer, **US 2826532**, Siggia, **US 2900305** (1955, 1958, and 1959, all to GAF). Prepn, history and use: Shelanski, Shelanski, *J. Int. Coll. Surgeons* **25,** 727 (1956).

Yellowish-brown, amorphous powder with slight characteristic odor. Aq solns have a pH near 2 and may be made more neutral (but less stable) by the addition of sodium bicarbonate. Sol in alc, water. Practically insol in chloroform, carbon tetrachloride, ether, solvent hexane, acetone. Solns do not give the Lugolian starch test when freshly prepared.

THERAPEUTIC: Anti-infective (topical).

THERAPEUTIC (VET.): Anti-infective (topical).

7785. PPACK. [71142-71-1] D-Phenylalanyl-*N*-[4-[(aminoiminomethyl)amino]-1-(chloroacetyl)butyl]-L-prolinamide; D-phenylalanyl-prolyl-arginine chloromethyl ketone; FPRMeCl. $C_{21}H_{31}ClN_6O_4$; mol wt 450.97. C 55.93%, H 6.93%, Cl 7.86%, N 18.64%, O 10.64%. Selective irreversible inhibitor of thrombin: C. A. Kettner, J. W. Shaw, *Thromb. Res.* **14,** 969 (1979). Synthesis: J. W. Shaw, C. A. Kettner, **US 4318904** (1982 to Research Corp.). Anticoagulant and antithrombotic activity: J. Hauptman, J. Markwardt, *Thromb. Res.* **20,** 317 (1980). *In vivo* studies: D. Collen *et al., J. Lab. Clin. Med.* **99,** 76 (1982).

Clinical Cancer Research

Phase I and Pharmacokinetic Study of ABI-007, a Cremophor-free, Protein-stabilized, Nanoparticle Formulation of Paclitaxel

Nuhad K. Ibrahim, Neil Desai, Sewa Legha, et al.

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EXHIBIT 3

Phase I and Pharmacokinetic Study of ABI-007, a Cremophor-free, Protein-stabilized, Nanoparticle Formulation of Paclitaxel[1]

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Departments of Breast Medical Oncology [N. K. I., R. L. T., E. R., G. N. H.], Melanoma/Sarcoma [S. L., S. E. R., A. B., J. A. E.], and Ophthalmology [B. E.], The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030, and American Bioscience, Inc., Santa Monica, California 90403 [N. D., P. S-S.]

ABSTRACT

Purpose: **ABI-007 is a novel Cremophor-free, protein-stabilized, nanoparticle formulation of paclitaxel. The absence of Cremophor EL may permit ABI-007 to be administered without the premedications used routinely for the prevention of hypersensitivity reactions. Furthermore, this novel formulation permits a higher paclitaxel concentration in solution and, thus, a decreased infusion volume and time. This Phase I study examines the toxicity profile, maximum tolerated dose (MTD), and pharmacokinetics of ABI-007.**

Experimental Design: **ABI-007 was administered in the outpatient setting, as a 30-min infusion without premedications. Doses of ABI-007 ranged from 135 (level 0) to 375 mg/m^2 (level 3). Sixteen patients participated in pharmacokinetic studies.**

Results: **Nineteen patients were treated. No acute hypersensitivity reactions were observed during the infusion period. Hematological toxicity was mild and not cumulative. Dose-limiting toxicity, which occurred in 3 of 6 patients treated at level 3 (375 mg/m^2), consisted of sensory neuropathy (3 patients), stomatitis (2 patients), and superficial keratopathy (2 patients). The MTD was thus determined to be 300 mg/m^2 (level 2). Pharmacokinetic analyses revealed paclitaxel C_{max} and area under the curve$_{inf}$ values to increase linearly over the ABI-007 dose range of 135–300 mg/m^2. C_{max} and area under the curve$_{inf}$ values for individual patients correlated well with toxicity.**

Conclusions: **ABI-007 offers several features of clinical interest, including rapid infusion rate, absence of requirement for premedication, and a high paclitaxel MTD. Our results provide support for Phase II trials to determine the antitumor activity of this drug.**

INTRODUCTION

Paclitaxel is a chemotherapeutic agent with a wide spectrum of antitumor activity when used as monotherapy or in combination chemotherapy regimens (1). The drug is used extensively in the treatment of advanced carcinomas of the breast, ovary, head and neck, and lung. Research into its activity in prostate cancer and urothelial tumors is ongoing as well. On the basis of early reports suggesting a dose-response phenomenon (2, 3), and in keeping with standard medical oncology practice, attempts are generally made to maintain paclitaxel doses at or near the MTD.[3] Several schedules of administration have been studied, each demonstrating a slightly different toxicity profile. Short infusions of 1–3 h result in peripheral neuropathy as a dose-limiting toxicity, whereas longer, continuous infusion schedules produce a higher incidence of neutropenia (2, 4–6). Other common side effects include alopecia, mucositis, arthralgias, myalgias, and mild nausea.

The paclitaxel preparation in clinical use (Taxol; Bristol-Myers Squibb, Princeton, NJ) is formulated in the nonionic surfactant Cremophor EL (polyoxyethylated castor oil) and ethanol to enhance drug solubility (7). Cremophor EL may add to paclitaxel's toxic effects by producing or contributing to the well-described hypersensitivity reactions that commonly occur during infusion, affecting 25–30% of treated patients (8, 9). To minimize the incidence and severity of these reactions, premedication with histamine 1 and 2 blockers, as well as glucocorticoids (usually dexamethasone), has become standard practice (10). The cumulative side effects of dexamethasone used as a premedication may add to treatment-related morbidity and, in some instances, result in early discontinuation of therapy. Cremaphor EL may also contribute to chronic paclitaxel toxic effects, such as peripheral neuropathy (11). An additional problem arising from the Cremophor and ethanol solvent is the leaching of plasticizers from PVC bags and infusion sets in routine clinical use (12). Consequently, Taxol must be prepared and administered in either glass bottles or non-PVC infusion systems and with in-line filtration. These problematic issues have spurred interest in the development of taxanes with improved solubility in aqueous solutions (13).

ABI-007 is a novel Cremophor-free formulation of paclitaxel (14). It is prepared by high-pressure homogenization of paclitaxel in the presence of human serum albumin, resulting in a nanoparticle colloidal suspension. Like Taxol, ABI-007 dos-

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[1] Supported by American Bioscience, Inc., Santa Monica, CA.
[2] To whom requests for reprints should be addressed, at Department of Molecular and Cellular Oncology, Box 79, M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030. Phone: (713) 792-8990; Fax: (713) 794-0209; E-mail: jaellerh@mail.mdanderson.org.

[3] The abbreviations used are: MTD, maximum tolerated dose; ANC, absolute neutrophil count; AUC, area under the curve; CL, clearance; PVC, polyvinyl chloride.

EXHIBIT 3

Table 1 Dose levels

Level	Dose (mg/m^2)	No. patients entered	No. cycles
0	135	4	6
1	200	3	38
2	300	6	35
3	375	6	17

age is determined by the paclitaxel content of the formulation, making direct comparison of the two drugs possible. ABI-007 can be reconstituted in normal saline at concentrations of 2–10 mg/ml, compared with 0.3–1.2 mg/ml for Taxol. Thus, the volume and time required for administration is reduced. In the absence of Cremophor EL, the risk of hypersensitivity reactions should decrease significantly, and patients receiving ABI-007 might thus avoid premedication. Moreover, there is no danger of leaching plasticizers from infusion bags or tubing, and conventional PVC infusion systems may be safely used.

To explore the potential clinical utility of ABI-007, we have conducted a Phase I study of this drug for patients with advanced solid tumors. The objectives of this trial were to determine the toxic effects, MTD, and pharmacokinetic profile of this unique paclitaxel preparation.

PATIENTS AND METHODS

Patient Eligibility and Evaluation on Study. Eligible patients included those with a diagnosis of an advanced solid tumor, having failed standard therapy. Requirements included a Zubrod performance status of 0–3, an expected survival of >6 weeks, hemoglobin ≥ 9 g/dl, ANC ≥ 1,500/mm^3, platelet count ≥ 100,000/mm^3, serum creatinine < 2 mg/dl, and serum bilirubin < 1.5 mg/dl. Patients with prior exposure to taxanes were eligible for the study.

Pretreatment evaluations included a complete blood count with differential and platelet count, serum chemistry profile, chest radiograph, and electrocardiogram. Baseline imaging studies and serum tumor marker levels were obtained at the discretion of the treating physician. Brain imaging by computerized tomography or magnetic resonance imaging was required for patients with symptoms suggestive of central nervous system involvement. Evaluations performed during the study included a complete blood count with differential and platelet count at least once weekly and a chemistry profile prior to each course. Restaging was performed after every 2nd or 3rd cycle of therapy. Patients were removed from the study for progression of disease, unacceptable toxicity, or at the patient's request.

Study Design. This Phase I study was conducted at The University of Texas M. D. Anderson Cancer Center and was approved by the M. D. Anderson Institutional Review Board. Informed consent was obtained from all subjects. Toxicity was graded according to National Cancer Institute Common Toxicity Criteria. Dose levels of ABI-007 are shown in Table 1. Dose escalation followed the standard "3 + 3" rule. Briefly, 3 patients were accrued at the starting dose level. If no toxic effects greater than grade 2 were observed, 3 patients were entered at the next dose level. If, at any level, one of the first 3 patients experienced a grade 3 or 4 toxic effect, 3 additional patients were entered at that dose level. The MTD was defined as one dose level below that at which ≥2 patients experienced grade 3 or 4 toxic effects. Six patients were to be treated at the MTD. Patients were permitted to escalate to the next higher dose level if no significant toxic effects were observed after the first 2 cycles of therapy. Patients with toxicity greater than grade 2 were permitted to reduce dosage by one dose level and remain on therapy at the discretion of the treating physician.

Treatment. ABI-007 was supplied by American Bioscience, Inc. (Santa Monica, CA). All therapy was administered in the outpatient treatment center of the M. D. Anderson Cancer Center, with the exception of patients participating in pharmacokinetic studies, which required an overnight hospital stay. The prescribed dose of ABI-007 was prepared in 100–150 ml of 0.9% saline. The drug was administered i.v. without in-line filtration and without premedication. For the first 3 patients on study, the total dose of ABI-007 was administered at a rate of 1.4 mg/kg/h or roughly over 3 h. If no acute hypersensitivity reactions were noted, the remainder of the patients were to receive treatment over 30 min. One cycle of therapy was 21 days.

Pharmacokinetic Studies. Pharmacokinetic studies were performed in 16 patients, with at least 3 patients representing each dose level. Whole blood samples of 5 ml each were taken to determine the pharmacokinetics of ABI-007 at 13 time points: 0, 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 12, 18, 24, and 48 h. Paclitaxel was extracted from whole blood samples using protein precipitation with acetonitrile, followed by solid phase extraction. The sample extracts were analyzed for paclitaxel using liquid chromatography atmospheric pressure ionization tandem mass spectrometry. The limit of quantitation for paclitaxel is 5 ng/ml, and the range of reliable response is 5–1000 ng/ml.

Pharmacokinetic parameters were determined from each patient's whole blood/plasma paclitaxel concentration profile. Analysis was performed by the noncompartmental routine using WinNonlin software (Pharsight Corp., Mountain View, CA). The peak or maximum paclitaxel concentration (C_{max}) and the corresponding peak time (t_{max}) were observed values. The elimination constant ($\lambda 12_z$) was obtained by log-linear regression analysis of the terminal phase of the whole blood/plasma concentration *versus* time profile. The elimination half-life ($T_{1/2}$) was determined by taking the ratio of natural log of 2 and $\lambda 12_z$. The AUC from time 0 to time infinity (AUC_{inf}) was obtained by summation of AUC_{last} (AUC from time 0 to last measurable concentration, calculated by the linear trapezoidal rule) and AUC_{ext} (extrapolated area, estimated by taking the ratio between the last measurable concentration and $\lambda 12_z$). The dose area relationship (*i.e.*, total ABI-007 dose divided by AUC_{inf}) was used to determine total body CL. The volume of distribution (V_z) was determined by taking the ratio between CL and $\lambda 12_z$.

Descriptive statistics (mean, median, SD, coefficient of variation, maximum, and minimum) were computed for pertinent pharmacokinetic parameters by ABI-007 dose. Regression analysis of mean AUC_{inf} *versus* dose was performed to gain an appreciation of pharmacokinetic linearity, if evident, for the dose range evaluated in this trial. Differences in the means of C_{max} and AUC_{inf} between groups of patients were analyzed for significance using a two-tailed, two-sample *t* test. Pearson's

Table 2 Patient characteristics

	No. (%)
Enrolled	20
Eligible	19
Age (yr)	
Median	50
Range	33–83
Performance status (Zubrod)	
0	2 (10)
1	14 (74)
2	3 (16)
Gender	
Female	16 (84)
Male	3 (16)
Malignancy	
Breast cancer	13 (68)
Melanoma	6 (32)
Prior treatment	
Chemotherapy	19 (100)
Immunotherapy	6 (32)
Radiotherapy	15 (79)

correlation coefficient was used to examine the correlation between degree of myelosuppression and C_{max} or AUC_{inf}.

RESULTS

Patients. Twenty patients were enrolled in the trial. One of these chose not to be treated after signing an informed consent. Therefore, 19 patients received drug and were evaluable for toxic effects. Patient characteristics are summarized in Table 2.

Treatment and MTD Determination. All treatment was administered without dexamethasone or histamine 1 or 2 blockers. The first 3 patients received infusions of ABI-007 over 2–3 h. No hypersensitivity reactions were observed. Therefore, all subsequent infusions were administered over 30 min. Even at the faster infusion rate, there were no instances of acute hypersensitivity to the ABI-007 preparation.

Three patients were entered initially at level 0, receiving 135 mg/m^2 over 3 h. One of these experienced progression of disease over the next several weeks, with rapid clinical deterioration, making it difficult to ascertain toxic effects of ABI-007 in this individual. To verify toxicity data at this dose level and ascertain the safety of administering the drug over a short infusion period, a 4th patient was entered at level 0 and was the first patient to receive drugs over 30 min. There were no instances of grade 3 or 4 toxicity observed at dose levels 0 or 1 (200 mg/m^2). At dose level 2 (300 mg/m^2), 1 of the first 3 patients developed grade 3 sensory neuropathy. Three more patients were accrued at this level, with no additional observations of dose-limiting toxicity. At dose level 3 (375 mg/m^2), during the 1st cycle of treatment, one of the first 3 patients experienced grade 3 sensory neuropathy, grade 3 stomatitis, and a visual disturbance diagnosed as superficial keratopathy, also grade 3. An additional 3 patients were accrued at level 3. One patient from this second cohort experienced a similar spectrum of grade 3 toxic effects, including sensory neuropathy, stomatitis, and superficial keratopathy; this patient developed grade 3 vomiting and diarrhea and thrombocytopenia as well. An additional case of sensory neuropathy, this time as an isolated grade 3 toxic effect, was observed in a 3rd patient at level 3. The study was thus terminated. The MTD for ABI-007 administered as a 30-min infusion every 21 days, as determined by this study, was 300 mg/m^2. The dose-limiting toxic effects were sensory neuropathy, stomatitis, and superficial keratopathy. Specific toxic effects are described below.

Table 3 Median absolute neutrophil and platelet nadirs by dose level

Dose level	ANC nadir × 10^3/mm^3 (range)	Platelet nadir × 10^3/mm^3 (range)
0	2.229 (1.850–5.040)	204 (174–292)
1	1.845 (0.586–3.729)	197 (118–270)
2	0.960 (0.264–3.680)	200 (105–609)
3	0.966 (0.018–1.804)	173 (25–251)

Hematological Toxicity. Hematological toxicity was dose dependent but remained modest throughout the study (Table 3). Of the 96 treatment cycles administered, only 7 (7.3%) resulted in an ANC nadir < 500/mm^3, 6 of which occurred above the MTD at dose level 3. There was one hospital admission for febrile neutropenia. In only one case did the platelet count drop below 75,000/mm^3. The patient, who was found to have a platelet nadir of 25,000/mm^3 during her 1st cycle of therapy at level 3, also developed a constellation of grade 3 nonhematological toxic effects. This was the only individual who required a platelet transfusion during the study. No patients received growth factors for granulocyte support.

Nonhematological Toxicity. Table 4 summarizes the nonhematological toxic effects observed during the first 2 cycles of therapy at each dose level. The majority of these were grades 1 and 2; no patient manifested grade 4 toxicity. Nausea, vomiting, and muscle and joint aches were common but mild. Skin toxicity was also mild, consisting of dry skin or localized vesicular or pustular rash. Alopecia was universal. Peripheral neuropathy, absent at the lower dose levels, was common with higher doses, appearing in 11 of 12 patients treated at levels 2 and 3. The neuropathy occurred in a typical stocking/glove distribution and was manifested by numbness or pain. Six patients with peripheral neuropathy developed peri-oral numbness as well. As described above, the most severe nonhematological adverse effects occurred in 2 patients at dose level 3, consisting of a complex of peripheral neuropathy, stomatitis, and superficial keratopathy, all grade 3.

A variety of ocular side effects was observed, the severity of which appeared to be dose dependent. One patient, entered at level 0, complained of dry eyes but noted no visual disturbance. No ocular complaints were registered by patients treated at level 1. Four patients developed ocular toxicity at level 2. One noted intermittent "smoky" vision, and another experienced blurred vision, both occurring with cycle 1 and both presenting as grade 1. Two other patients at dose level 2 noted "flashing lights" and photosensitivity during their third course of treatment. One went on to develop grade 2 superficial keratopathy during course 4. The other experienced a reversible decrease in visual acuity without specific abnormalities on ophthalmologic exam. At level 3, 2 patients complained of mild dry eyes throughout

Table 4 Nonhematologic toxicity by dose level[a]

Toxicity	Level 0 (n = 4)		Level 1 (n = 3)		Level 2 (n = 6)		Level 3 (n = 6)	
	Grade 1 or 2	Grade 3	Grade 1 or 2	Grade 3	Grade 1 or 2	Grade 3	Grade 1 or 2	Grade 3
Sensory neuropathy	0	0	0	0	4	1	3	3
Ocular	1	0	0	0	2	0	2	2
Stomatitis	0	0	1	0	4	0	3	2
Nausea	1	0	1	0	3	0	4	1
Vomiting	1	0	1	0	0	0	2	1
Diarrhea	1	0	2	0	3	0	1	1
Arthralgia/myalgia	3	0	3	0	4	0	4	1
Skin	0	0	0	0	5	0	2	0
Fever (non-neutropenic)	0	0	0	0	2	0	3	0

[a] Expressed as the number of patients experiencing the toxic effect during the first two cycles of treatment.

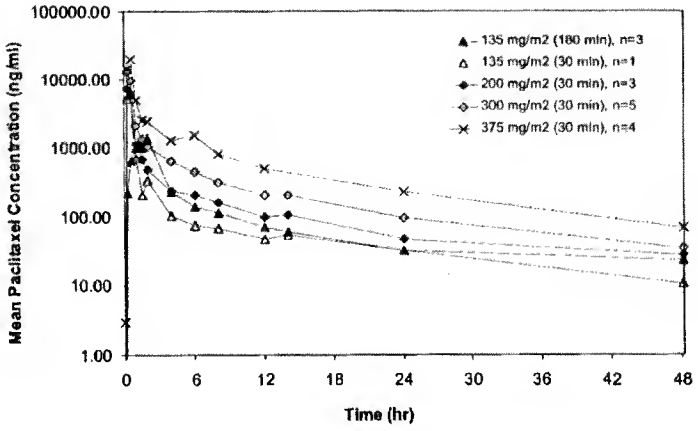

Fig. 1 Pharmacokinetic profile of ABI-007 showing mean whole blood paclitaxel concentrations at increasing doses of ABI-007 *versus* time. All infusions were given over 30 min except for the first 3 patients who received 135 mg/m^2 over 180 min.

therapy but did not experience visual disturbances. Two other patients at dose level 3 developed grade 3 superficial keratopathy during their 1st cycle of treatment, as described above. All cases of keratopathy received full ophthalmologic evaluation, and all resolved with the use of topical lubricating drops and ointments. No patient developed a permanent loss of vision or experienced any other permanent ocular sequellae.

Occurrences of new types of toxic effects after the first 2 cycles of therapy were rare. Furthermore, it was uncommon for toxic effects to increase in grade after the first 2 treatment cycles. Therefore, cumulative toxicity did not appear to be a significant problem.

Response. Partial responses were observed in two breast cancer patients, both of whom had prior exposure to Taxol. The first patient, entered at dose level 2, experienced a 68% decrease in the size of pulmonary metastases. This response lasted a total of 15 months, including 9 months after discontinuation of therapy for toxicity. The 2nd patient, who was also treated at dose level 2, had significant improvement in soft tissue disease involving the chest wall. Because of toxic effects, she was taken off treatment on the date of her response. Disease progression was noted 6 weeks later.

Pharmacokinetic Studies. Sixteen of the 19 patients entered into the study contributed analyzable pharmacokinetic profiles. Three of these received ABI-007 as a 180-min infusion; the remaining 13 were treated over 30 min. A semilog plot of the mean values of the whole blood paclitaxel concentration for each dose level *versus* time is shown in Fig. 1. The maximum paclitaxel concentrations were observed at the termination of ABI-007 infusion; the decline from maximum was biphasic.

A summary of the pharmacokinetic parameter values derived by noncompartmental methods is shown in Table 5. The pharmacokinetics of ABI-007 administered over 30 min appeared to be linear across the three lower dose levels, which included the MTD (Fig. 2). Calculations from the data in Table 5 reveal a 2.2-fold increase in C_{max} and a 2.7-fold increase in AUC_{inf} over the 2.2-fold increase in dose from 135 to 300 mg/m^2. The decline in CL estimates over this range is 0.8-fold (16.1%). If the highest dose level of 375 mg/m^2 is included, nonlinearity becomes evident (Fig. 2). Individual C_{max} and AUC_{inf} values *versus* dose are shown in Fig. 3, *a* and *b*, respectively.

The group of 13 patients who received 30-min infusions and for whom pharmacokinetic profiles were obtained included 3 who experienced grade 3 nonhematological toxic effects (neuropathy with or without stomatitis and keratopathy). The C_{max} and AUC_{inf} for these 3 patients relative to those of the remaining 10 patients are plotted in Fig. 4. The differences in mean C_{max}

Table 5 Summary of noncompartmental pharmacokinetic parameters, mean (% coefficient of variation) values by dose[a]

Dose mg/m^2	Infusion duration min	*n*	C_{max} ng/ml	AUC_{inf} ng/h/ml	Half-life h	CL liter/h/m^2	V_z liter/m^2
135	180	3	1392 (30)	5654 (42)	12.9 (60)	27.4 (45)	418 (32)
135	30	1	6100	6427	14.6	21.1	442
200	30	3	7757 (35)	9613 (20)	13.4 (67)	21.4 (21)	384 (64)
300	30	5	13520 (7)	17610 (21)	14.6 (14)	17.7 (22)	370 (23)
375	30	4	19350 (15)	35805 (40)	13.2 (12)	11.9 (42)	236 (54)

[a] *n*, number of patients; C_{max}, maximum or peak concentration; AUC_{inf}, area under the whole blood/plasma concentration-time curve from time 0 to time infinity; CL, total body clearance; V_z, volume of distribution.

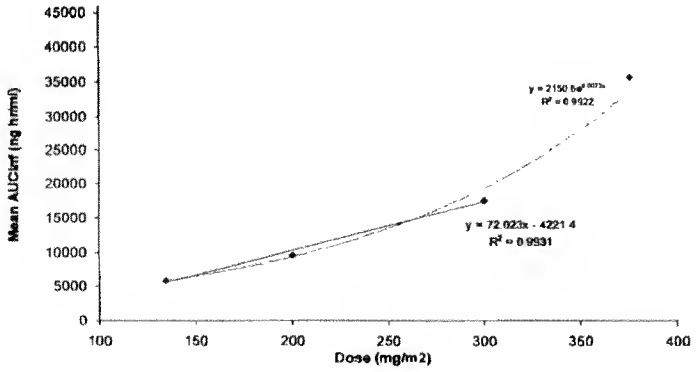

Fig. 2 Correlation between the mean AUC_{inf} and dose level. The data have been fit using a linear regression and an exponential regression function.

and mean AUC_{inf} between the two groups were significant ($P = 0.034$ and 0.007, respectively). The effect of ABI-007 exposure on myelosuppression was also examined in this group of patients. The percentage of decrease in ANC from baseline to nadir was found to correlate positively with both C_{max} ($r = 0.610$, $P = 0.027$) and AUC_{inf} ($r = 0.614$, $P = 0.025$).

DISCUSSION

This clinical trial was conducted to examine the pharmacokinetic properties and spectrum of toxic effects associated with ABI-007. Because ABI-007 is not formulated in a Cremophor-containing solvent, we anticipated that hypersensitivity reactions would be diminished or absent. Our results show that ABI-007 can indeed be administered safely as a short infusion without dexamethasone or antihistamine premedication. Thus, when considering the process of drug administration, ABI-007 appears to offer advantages in terms of safety (avoidance of hypersensitivity reactions), morbidity (avoidance of dexamethasone premedication), and patient convenience and comfort (less time spent in the treatment center). These advantages could ultimately translate into an overall decrease in cost of therapy.

It must be pointed out that, although the absence of Cremophor is clearly desirable with respect to toxicity, this same compound has been proposed to enhance the efficacy of cytotoxic drugs through reversal of the multidrug resistance phenotype (15). Plasma concentrations of Cremophor attainable during Taxol infusions are sufficient to inhibit P-glycoprotein effects *in vitro* (16). However, there have been questions raised as to whether these Cremophor concentrations are relevant to solid tumors, as pharmacokinetic studies demonstrate the compound's distribution to be limited to the central plasma compartment (17). This issue should be clarified with the completion of ongoing Phase II trials of ABI-007. If the response rate of ABI-007 is not less than that of Taxol and if responses are seen in patients who are previous taxane failures, the therapeutic contribution of Cremophor to paclitaxel can be considered negligible.

In terms of treatment-related toxicity, a lower incidence of myelosuppression was observed than that which we anticipated based on the dose of paclitaxel administered. In this regard, hematological toxicity was mild and played virtually no role in dose and treatment decisions made in this trial. Although direct comparisons to Taxol administered at this dose range and schedule are not possible, the myelosuppression induced by ABI-007 appeared to be similar to or less severe than that reported for 1-h Taxol infusions at lower doses (18). Otherwise, the spectrum of toxic effects produced by ABI-007 resembled that of high-dose short-infusion Taxol reported in early Phase I trials, with sensory neuropathy and mucositis becoming dose limiting (19, 20). A third dose-limiting toxic effect, superficial keratopathy, was also observed. We were unable to find any prior report of superficial keratopathy as a consequence of paclitaxel administration. In our Phase I trial, this side effect appeared to be related to dose and presented at the level of grade 3 only above the MTD, at a dose of 375 mg/m^2. Superficial keratopathy secondary to ABI-007 was similar to that most commonly recognized in association with 1-β-D-arabinofuranosylcytosine, although any type of ocular surface irritation, including dry eye syn-

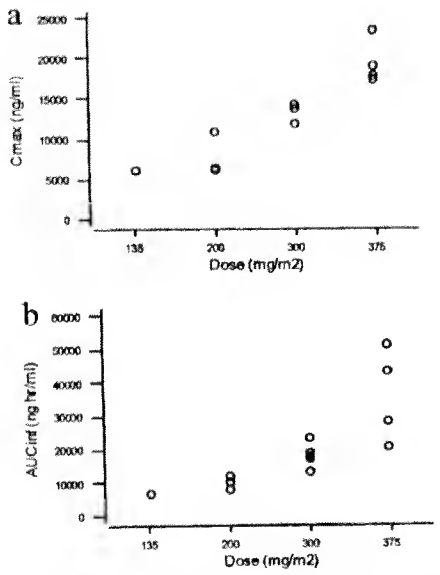

Fig. 3 Individual values of C_{max} (*a*) and AUC_{inf} (*b*) *versus* dose for patients receiving 30-min infusions of ABI-007.

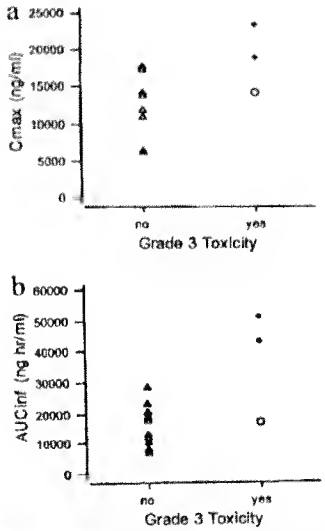

Fig. 4 Individual values of C_{max} (*a*) and AUC_{inf} (*b*) for patients who experienced grade 3 nonhematological toxic effects ("yes") and those who did not ("no"). In the "yes" category, the solid diamond symbols (◆) represent 2 patients with multiple grade 3 toxicities, whereas the open circle (○) represents the patient with only grade 3 neuropathy.

drome, can result in similar corneal findings (21). Other ocular complications of taxane therapy have been reported. The most common adverse ocular effects of Taxol are photopsia and blurred vision, usually reported by patients during the infusion period (22, 23). Cases of optic nerve disturbances have also been documented (24). Cases of grade 2 conjunctivitis necessitating dose reduction and treatment delay have been reported during weekly therapy with docetaxel (25). Similar to our findings, reported ocular effects from paclitaxel have been noted only at higher doses and are usually transient. Although all cases of keratopathy in this study resolved completely and without permanent sequellae, in the ensuing Phase II trial, patients will be aggressively monitored for the development of ophthalmologic abnormalities.

Pharmacokinetic analysis of ABI-007 revealed interesting similarities and differences relative to Taxol, based on published data. Disappearance from the blood is biphasic for both drugs (19). ABI-007 displays linear pharmacokinetics over the clinically relevant dose range of 135–300 mg/m^2; over a similar dose range, Taxol AUC_{inf} is nonlinear (26–28). In comparing the AUC_{inf} values of ABI-007 infused over 30 min to those reported for Taxol infused over 1 or 3 h, ABI-007 in general showed lower AUC_{inf} values over a similar range of doses (26–28). Although several explanations are possible for the differences in AUC_{inf}, it is reasonable to hypothesize that ABI-007 may be distributed more rapidly out of the vascular compartment, a suggestion supported by the difference in formulation between the two drugs. A substantial amount of solvent (Cremophor/ethanol) is infused with Taxol, and the partition of paclitaxel from the vascular compartment to the tissues may thus be relatively slow. In contrast, ABI-007 is formulated with human serum albumin at a concentration of 3–4%, similar to the concentration of albumin in the blood. Because paclitaxel has a very limited solubility in an aqueous albumin solution (<30 μg/ml), it may partition more efficiently into the tissues in the case of ABI-007. Furthermore, lipid, macromolecular, and nanoparticle drug carriers have been known to preferentially accumulate in tumor beds and tissues in what is known as enhanced permeation and retention effect (29). These factors may facilitate the partition of ABI-007 into tissues.

The MTD of ABI-007 was found in this study to be 300 mg/m^2 when given as a short infusion on a 21-day cycle. Although the usual dose range for Taxol is 135–200 mg/m^2, doses as high as 250 mg/m^2 are occasionally administered. Therefore, the MTD established by this trial represents a moderate increase over that of Taxol. The issue of whether one can achieve uniform and repeated dosing of ABI-007 at the MTD will need to be addressed in Phase II trials.

In conclusion, ABI-007 appears to represent an improvement in paclitaxel formulation in that it can be administered rapidly and safely without the risk of hypersensitivity reactions, eliminating the need for steroid and antihistamine premedication. Furthermore, the increased MTD and favorable toxicity profile of ABI-007 may ultimately prove advantageous in terms of rate and quality of response. Although several interesting pharmacokinetic properties were noted for ABI-007, the small number of patients in this study renders comparisons with Taxol preliminary, and additional studies will need to be conducted to fully appreciate differences in pharmacokinetic behavior. The partial responses seen in 2 patients with prior exposure to Taxol are encouraging and support a continued effort to explore the

spectrum of activity for this drug. We are currently conducting a Phase II trial of ABI-007 for patients with metastatic breast cancer to establish the antitumor activity of this novel paclitaxel formulation.

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REFERENCES

1. Crown, J., and O'Leary, M. The taxanes: an update. Lancet, *355:* 1176–1178, 2000.

2. Eisenhauer, E. A., ten Bokkel Huinink, W. W., Swenerton, K. D., Gianni, L., Myles, J., van der Burg, M. E. L., Kerr, I., Vermorken, J. B., Buser, K., Colombo, N., Bacon, M., Santabarbara, P., Onetto, N., Winograd, B., and Canetta, R. European-Canadian randomized trial of paclitaxel in relapsed ovarian cancer: high-dose *versus* low-dose and long *versus* short infusion. J. Clin. Oncol., *12:* 2654–2666, 1994.

3. Nabholtz, J. M., Gelmon, K., Bontenbal, M., Spielmann, M., Catimel, G., Conte, P., Klaassen, U., Namer, M., Bonneterre, J., Fumoleau, P., and Winograd, B. Multicenter randomized comparative study of two doses of paclitaxel in patients with metastatic breast cancer. J. Clin. Oncol., *14:* 1858–1867, 1996.

4. Smith, R. E., Brown, A. M., Mamounas, E. P., Anderson, S. J., Lembersky, B. C., Atkins, J. H., Shibata, H. R., Baez, L., DeFusco, P. A., Davila, E., Tipping, S. J., Beardea, J. D., and Thirlwell, M. P. Randomized trial of 3-hour *versus* 24-hour infusion of high-dose paclitaxel in patients with metastatic or locally advanced breast cancer: National Surgical Adjuvant Breast and Bowel Project Protocol B-26. J. Clin. Oncol., *17:* 3403–3411, 1999.

5. Wiernik, P. H., Schwartz, E. L., Einzig, A., Strauman, J. J., Lipton, R. B., and Dutcher, J. P. Phase I trial of taxol given as a 24-hour infusion every 21 days: responses observed in metastatic melanoma. J. Clin. Oncol., *5:* 1232–1239, 1987.

6. Socinski, M. A., Mudd, P. N., Radomski, K. M., Steagall, A., Lawrence, P., Bernard, S., Lefrent, S. P., Gonzalez, P., and Brouwer, K. L. R. Phase I trial of a 96 h paclitaxel infusion with filgrastim support in refractory solid tumor patients. Anticancer Drugs, *9:* 611–619, 1998.

7. Dorr, R. T. Pharmacology and toxicology of Cremophor EL diluent. Ann. Pharmacother., *28:* S11–S14, 1994.

8. Weiss, R. B., Donehower, R. C., Wiernik, P. H., Ohnuma, T., Gralla, R. J., Trump, D. L., Baker, J. R., Jr., Van Echo, D. A., Von Hoff, D. D., and Leyland-Jones, B. Hypersensitivity reactions from taxol. J. Clin. Oncol., *8:* 1263–1268, 1990.

9. Rowinsky, E. K., and Donehower, R. C. Paclitaxel (taxol). N. Eng. J. Med., *332:* 1004–1014, 1995.

10. Finley, R. S., and Rowinsky, E. K. Patient care issues: the management of paclitaxel-related toxicities. Ann. Pharmacother., *28:* S27–S30, 1994.

11. Windebank, A. J., Blexrud, M. D., and de Groen, P. C. Potential neurotoxicity of the solvent vehicle for cyclosporine. J. Pharmacol. Exp. Ther., *268:* 1051–1056, 1994.

12. Waugh, W. N., Trissel, L. A., and Stella, V. J. Stability, compatibility, and plasticizer extraction of taxol (NSC-125973) injection diluted in infusion solutions and stored in various containers. Am. J. Hosp. Pharm., *48:* 1520–1524, 1991.

13. Hidalgo, M., Aylesworth, C., Hammond, L. A., Britten, C. D., Weiss, G., Stephenson J., Jr, Schwartz, G., Patnaik, A., Smith, L., Molpus, K., Felton, S., Gupta, E., Ferrante, K. J., Tortora, A., Sonnichsen, D. S., Skillings, J., and Rowinsky, E. K. Phase I and pharmacokinetic study of BMS-184476, a taxane with greater potency and solubility than paclitaxel. J. Clin. Oncol., *19:* 2493–2503, 2001.

14. Desai, N. P., Louie, L., Ron, N., Magdassi, S., and Soon-Shiong, P. Protein-based nanoparticles for drug delivery of paclitaxel. Trans. World Biomater. Congr., *1:* 199, 2000.

15. Woodcock, D. M., Jefferson, S., Linsenmeyer, M. E., Crowther, P. J., Chojnowski, G. M., Williams, B., and Bertoncello, I. Reversal of the multidrug resistance phenotype with cremophor EL, a common vehicle for water-insoluble vitamins and drugs. Cancer Res., *50:* 4199–4203, 1990.

16. Webster, L., Linsenmeyer, M., Millward, M., Morton, C., Bishop, J., and Woodcock, D. Measurement of cremophor EL following taxol: plasma levels sufficient to reverse drug exclusion mediated by the multidrug-resistant phenotype. J. Natl. Cancer Inst. (Bethesda), *85:* 1685–1690, 1993.

17. Sparreboom, A., Verweij, J., van der Burg, M. E., Loos, W. J., Brouwer, E., Vigano, L., Locatelli, A., de Vos, A. I., Nooter, K., Stoter, G., and Gianni, L. Disposition of cremophor EL in humans limits the potential for modulation of the multidrug resistance phenotype *in vivo*. Clin. Cancer Res., *4:* 1937–1942, 1998.

18. Hainsworth, J. D., and Greco, F. A. Paclitaxel administered by 1-hour infusion. Preliminary results of a phase I/II trial comparing two schedules. Cancer (Phila.), *74:* 1377–1382, 1994.

19. Wiernik, P. H., Schwartz, E. L., Strauman, J. J., Dutcher, J. P., Lipton, R. B., and Paietta, E. Phase I clinical and pharmacokinetic study of taxol. Cancer Res., *47:* 2486–2493, 1987.

20. Donehower, R. C., Rowinsky, E. K., Grochow, L. B., Longnecker, S. M., and Ettinger, D. S. Phase I trial of taxol in patients with advanced cancer. Cancer Treat. Rep., *71:* 1171–1177, 1987.

21. Ritch, P. S., Hansen, R. M., and Heuer, D. K. Ocular toxicity from high-dose 1-β-D-arabinofuranosylcytosine. Cancer (Phila.), *51:* 430–432, 1983.

22. Tan, W. W., and Walsh, T. Ocular toxicity secondary to paclitaxel in two lung cancer patients. Med. Pediatr. Oncol., *31:* 177, 1998.

23. Seidman, A. D., Barrett, S., and Canezo, S. Photopsia during 3-hour paclitaxel administration at doses ≥ 250 mg/m^2. J. Clin. Oncol., *12:* 1741–1742, 1994.

24. Al-Tweigeri, T., Nabholtz, J. M., and Mackey, J. R. Ocular toxicity and cancer chemotherapy. Cancer (Phila.), *78:* 1359–1373, 1996.

25. Burstein, H. J., Younger, J., Bunnell, C. A., Matulonis, U. A., Garber, J. E., Shulman, L. N., Parker, L. M., Scheib, R., Clarke, K., Fowler, K., Gelman, R., and Winer, E. P. Weekly docetaxel (Taxotere) for metastatic breast cancer: a phase II trial. Proc. Am. Soc. Clin. Oncol., *18:* 127, 1999.

26. Kearns, C. M. Pharmacokinetics of taxanes. Pharmacotherapy, *17:* 105S–109S, 1997.

27. Mross, K., Hollander, N., Hauns, B., Schumacher, M., and Maier-Lenz, H. The pharmacokinetics of a 1-h paclitaxel infusion. Cancer Chemother. Pharmacol., *45:* 463–470, 2000.

28. Gianni, L., Kearns, C. M., Giani, A., Capri, G., Vigano, L., Locatelli, A., Bonadonna, G., and Egorin, M. J. Nonlinear pharmacokinetics and metabolism of paclitaxel and its pharmacokinetic/pharmacodynamic relationships in humans. J. Clin. Oncol., *13:* 180–190, 1995.

29. Maeda, H., Wu, J., Sawa, T., Matsumura, Y., and Hori, K. Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review. J. Control Release, *65:* 271–284, 2000.

EXHIBIT 3

Technical Leaflet

MK 074 e
(868) July 1997 (Bn)

Register 8 + 14

Cremophor® EL

® = Registered trademark of
BASF Aktiengesellschaft

Emulsifying agent for the pharmaceuticals, cosmetics and feedstuffs industries; used in aqueous preparations of hydrophobic substances, e.g. fat-soluble vitamins and essential oils.

Fine Chemicals

BASF

EXHIBIT 4

Common names

Polyoxyethylenglyceroltriricinoleat 35 (DAC), Polyoxyl 35 Castor Oil (USP/NF).

Nature

Cremophor EL is a non-ionic solubilizer and emulsifier obtained by causing ethylene oxide to react with castor oil of German Pharmacopoeia (DAB 8) quality in a molar ratio of 35 moles to 1 mole.

Composition

The main component of Cremophor EL is glycerol-polyethylene glycol ricinoleate, which, together with fatty acid esters of polyethyleneglycol, represents the hydrophobic part of the product. The smaller, hydrophilic part consists of polyethylene glycols and ethoxylated glycerol.

Properties

Cremophor EL is a pale yellow, oily liquid that is clear at temperatures above 26 °C. It has a slight but characteristic odour and can be completely liquefied by heating to 26 °C. The hydrophilic-lipophilic balance (HLB) lies between 12 and 14.

Specification

Viscosity (Höppler) at 25 °C	700 – 850 mPa · s
Mass density at 25 °C	1.05 – 1.06 g/ml
Refractive index at 25 °C	1.465 – 1.475
Saponification value	63 – 72
Hydroxyl value	65 – 78
Iodine value	28 – 32
Acid value	≤ 2
Water content (K. Fischer)	≤ 3%
pH value of 10% aqueous solution	6 – 8
Sulfated ash	≤ 0.2%
Heavy metals (USP XX method)	≤ 10 ppm

Unless otherwise indicated, the values were determined according to the monograph "Polyoxyäthylenglyceroltriricinoleat 35" of the Deutscher Arzneimittelcodex and to the monograph "Polyoxyl 35 Castor Oil", USP/NF.

Solubility

Cremophor EL forms clear solutions in water. It is also soluble in ethyl alcohol, n-propyl alcohol, isopropyl alcohol, ethyl acetate, chloroform, carbon tetrachloride, trichloroethylene, toluene and xylene.

In contrast to that of anionic emulsifying agents, the solubility in water decreases with rising temperature. Thus, aqueous solutions become turbid at a certain temperature.

Cremophor EL is miscible with all other Cremophor grades and, on heating, with fatty acids, fatty alcohols and certain animal and vegetable oils. It is thus miscible with oleic and stearic acids, dodecyl and octadecyl alcohols, castor oil, and a number of lipid-soluble substances.

Stability

Cremophor EL in aqueous solutions is stable towards electrolytes, e.g. acids and salts, provided that their concentration is not too high. Mercury (II) chloride is an exception and forms a precipitate with the product.

Some organic substances may cause precipitation at certain concentrations, especially compounds containing phenolic hydroxyl groups, e.g. phenol, resorcinol and tannin.

Cremophor EL can be sterilized by heating in an autoclave for 30 minutes at 120 °C. It may thus acquire a deeper shade. During sterilization, Cremophor EL should not be heated together with substances that are strongly acidic or alkaline and would thus saponify it.

Application

Cremophor EL is recommended as a solubilizer and emulsifier in many different branches of industry. It is particularly suitable for the production of liquid preparations.

The degree to which the hydrophobic substance is distributed in the liquid depends largely on its properties and on the amount of Cremophor EL used. A rule of thumb is that, if Cremophor EL is present in excess, clear or opalescent liquids are obtained. However, if the proportion of Cremophor EL is reduced to, say 5 – 10%, expressed in terms of water-insoluble substance, conditions exist for the formation of an emulsion.

EXHIBIT 4

In aqueous solution, Cremophor EL emulsifies or solubilizes the fat-soluble vitamins A, D, E and K. In aqueous-alcoholic solutions, it very readily solubilizes essential oils. Other hydrophobic drugs can also be converted into aqueous solutions with Cremophor EL (e.g. Miconazole, Hexedetine, Clotrimazole, Benzocaine).

In order to ensure that the fat-soluble vitamins yield clear aqueous solutions, they must first be intimately mixed with the solubilizer. The preferred forms of vitamin A for this purpose are vitamin A palmitate with 1.7 million I.U./g or vitamin A propionate with 2.5 million I.U./g; and the preferred form of vitamin K is vitamin K_1 (phytomenadione).

An important factor is how the water-soluble substance is solubilized. Hence, a typical example, viz. the preparation of an aqueous vitamin A palmitate solution with 150000 I.U./ml, is described in detail below.

Vitamin A palmitate 1.7 million I.U./g	8.8 g
Cremophor EL	25.0 g
Water	ad 100 ml

The Cremophor EL is mixed with the vitamin and heated to 60–65 °C. The water, also heated to 60–65 °C, is intimately incorporated in the mixture by slowly stirring in. Initially, thickening occurs as a result of hydration and reaches a maximum when about half of the water has been added. On addition of the remaining water, the viscosity is reduced again. If the first half of the water is added too rapidly, an opalescent solution may be obtained.

The following three diagrams show that clear aqueous solutions of vitamin A palmitate, vitamin A propionate or vitamin E acetate can be obtained in very high concentrations with the aid of Cremophor EL. Concentrations refer to the finished solubilisates.

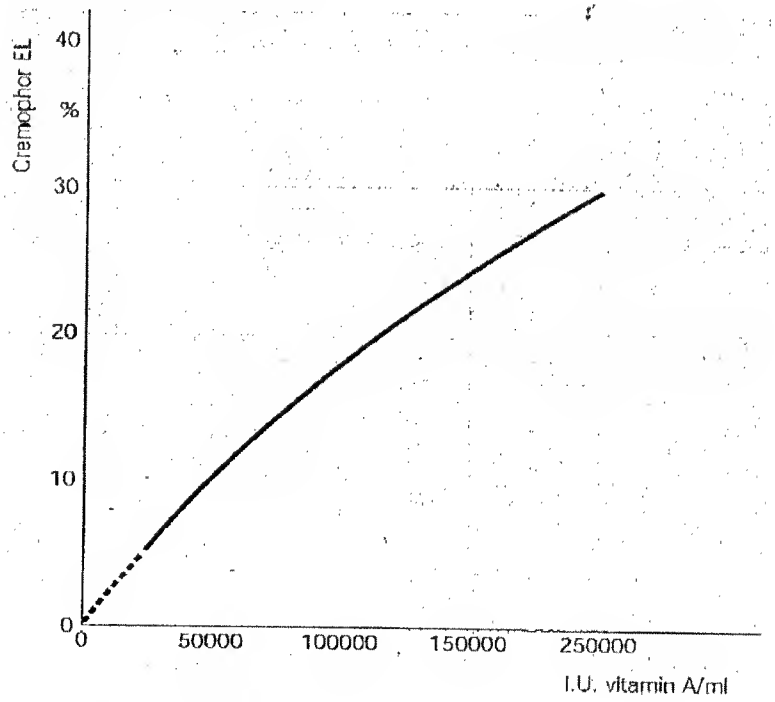

Fig. 1 Vitamin A palmitate

EXHIBIT 4

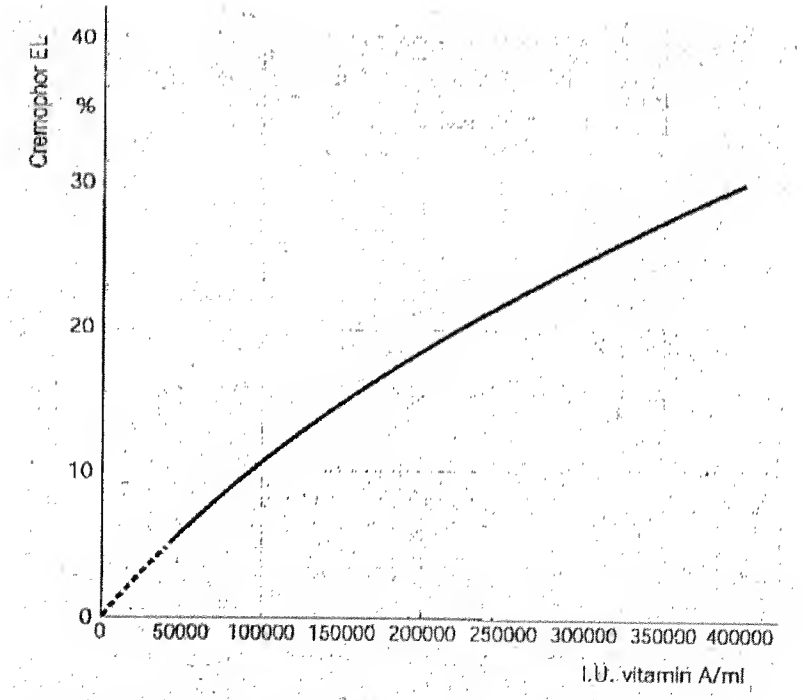

Fig. 2 Vitamin A propionate

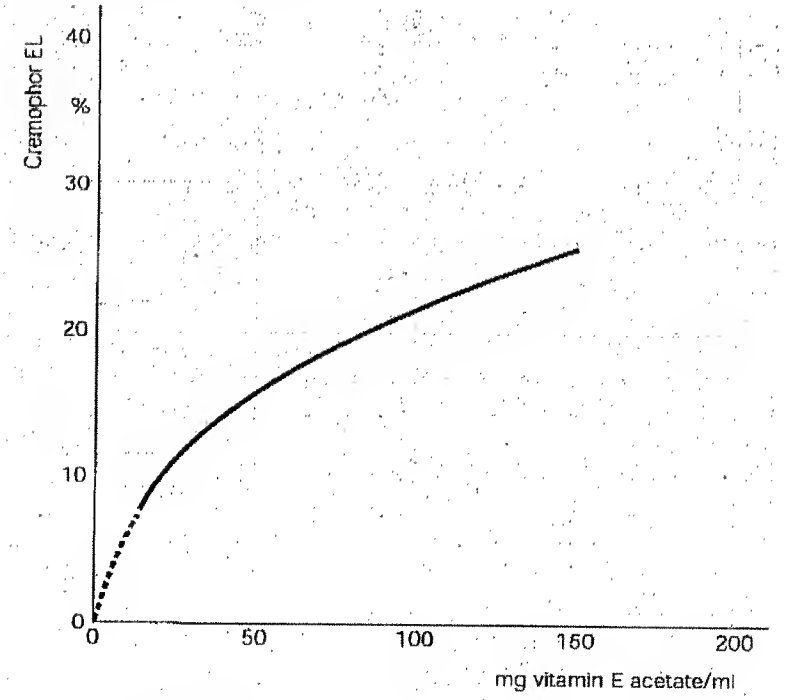

Fig. 3 Vitamin E acetate

EXHIBIT 4

The following amounts of other fat-soluble vitamins can be dissolved in a 6% solution of Cremophor EL:

ca. 200000 I.U. vitamin D_3/ml or
ca. 10 mg vitamin K_1/ml

As a rule, less Cremophor EL is required for mixtures of various vitamins.

The processing temperature and, in some cases, the amount of Cremophor EL required can be reduced by adding small amounts of polyethylene glycol (Lutrol® E 400), propylene glycol or glycerol. The stability of many solubilisates may be affected by light.

For reasons of taste, it is recommended that the hydrogenated and thus tastelese form, viz. Cremophor RH 40, be used for oral application in human medicine. The inherent odour of Cremophor EL can best be masked in many cases with banana aroma.

A solution of one part of azulene in about four parts of Cremophor EL can be infinitely diluted with water. In addition, Cremophor EL has proved to be a useful additive in the production of glycerol suppositories.

Cosmetics

In the cosmetics industry, Cremophor EL is used preferentially for solubilizing perfume oils and for emulsifying fatty substances, organic solvents, and additives. Cremophor EL is an outstanding solubilizer for aroma chemicals and ethereal oils in aqueous isopropyl or ethyl alcohol, provided that the alcohol concentration is 30 – 50%. In many cases, extremely small additions of Cremophor EL are adequate under these conditions, so that the inherent odour of the product is completely masked. The solubilizers Cremophor RH 40 and Cremophor RH 60, which are also highly efficient, are completely free from odour and taste.

For the production of completely clear solutions of perfume oil in aqueous alcohol, the perfume oil and the solubilizer should be dissolved together in concentrated alcohol, after which the water is added slowly.

Animal nutrition and veterinary medicine

By virtue of its good dispersing action, Cremophor EL enables nutritive and therapeutic substances to be assimilated more completely and thus renders them more effective. This fact is of particular interest for compounded feeds containing oils and fats. A special application of Cremophor EL is the production of cod-liver oil emulsions in veterinary medicine.

Physiological properties

Cremophor EL is tolerated extremely well, as tests with single and repeated oral doses and exposure tests on the skin and mucous membranes have shown.

Acute toxicity

LD 50 (7 days follow-up period):

Rat oral	>	6.4 ml/kg
Rabbit oral	>	10.0 ml/kg
Cat oral	>	10.0 ml/kg
Mouse I.v.		2.5 – 4 ml/kg
Rat percutaneous	>	4.0 ml/kg (maximum applicable dose)

No characteristic toxic symptoms were observed after oral doses or application to the skin, and no pathological changes of the inner organs were discernible with the naked eye during autopsy.

Acute inhalation toxicity

Cremophor EL is pratically non-volatile. In tests, rats have inhaled air saturated at 20 °C with the volatile components of the product for over eight hours without suffering any irritation of respiratory tract or any injury by absorption.

Irritation of skin and mucous membranes

Contact for more than 20 hours between the undiluted product and the highly sensitive skin on the backs and ears of white rabbits caused only slight or insignificant inflammation that disappeared rapidly.

This instillation of 0.05 ml of Cremophor EL in the rabbit's conjunctival sac only caused slight reddening of the conjunctiva that disappeared within a few hours. The application of a 50% aqueous solution of the product caused slight irritation and lachrymation, both of which disappeared rapidly; 30% aqueous solutions had no irritant effect.

EXHIBIT 4

Repeated application of a 50% solution of Cremophor EL in acetone with a brush to the skin of guinea-pigs produced inflammatory reactions at the affected parts but did not cause any sensitization. Intracutaneous injection of 0.05 or 0.1 ml of a 0.1% solution in physiological sodium chloride solution ten times on successive days to a guinea-pig did not cause sensitization.

Subacute toxicity

Repeated oral administration of Cremophor EL in doses of 0.5, 1.0, 2.5 and 5.3 ml/kg daily (5 times a week over four weeks) with the oesophageal sound to beagles did not cause any clinically detectable disorder except for soft faeces in some cases. In clinical-chemical and pathological-histological tests, the experimental animals did not show any pathological changes attributable to Cremophor EL.

Feeding tests

In six-month feeding tests carried out on rats and dogs with Cremophor EL in concentrations of up to 1%, the experimental animals showed no visible symptoms of poisoning, no impairment of feed ingestion or growth, no detectable disorders of the blood and urine, no organic malfunctions, no increase in weight of the organs, and no abnormal organic mutation that could be detected in pathological-histological tests (no-effect level).

Teratological effect

No teratological or embryotoxic effect of Cremophor EL (tested according to the FDA specifications: Guidelines for reproduction studies for safety evaluation of drugs for human use; 1966) after oral application of 10 and 5 ml/kg daily from the 6th to the 15th day post coitum with the oesophageal sound was observed in NMRI mice. Even the addition of 10% and 5% of Cremophor EL to the feed of pregnant Sprague-Dawley rats during the organogenesis period, i.e. day 0–20, had no embryotoxic or teratological effect.

Detailed toxicological test reports on Cremophor EL are available on request.

Effect on action of drugs

The fine degree of dispersion resulting from addition of Cremophor EL allows a drug to be absorbed more readily and increases its efficiency.

Cremophor EL promotes the penetration of a number of active substances and exerts either activating or inactivating effects on others, e.g. antibiotics. Therefore, before Cremophor EL preparations are used in practice, it is advisable to subject them to thorough pharmacological tests.

Cremophor EL is subjected to detailed quality control involving comprehensive chemical and physical tests. The individual production batches are not, however, subjected to biological tests. For this reason, all producers of Cremophor EL-preparations must carry out their own tests to check the suitability of the material used and the final preparations.

Cattle that have been subjected to parenteral treatment with certain vaccines or medicaments and subsequently injected with preparations containing Cremophor EL or similar solubilizers have displayed anaphylactoid reactions in isolated, exceptional cases. After the application of injections containing Cremophor EL to human beings, anaphylactoid reactions have sometimes been observed. For this reason, the health authorities in the Federal Republic of Germany and the U.K., for instance, have laid down hat the content of polyethoxylated castor oil in injections for parenteral application to human beings must be declared, and any possibility of side effects must be pointed out in the package circular. This is an aspect to which companies producing pharmaceuticals for human beings must pay particular attention.

After oral administration of preparations containing Cremophor EL, side effects of this kind have not been observed.

Packaging

Drums of 60 kg and 120 kg capacity.

Product number

00647/1/63

Safety Data Sheet

A Safety Data Sheet is available.

Storage

Cremophor EL should be stored in tightly closed containers and protected from light. Prolonged storage is not advisable unless the containers are completely full.

EXHIBIT 4

Note

The data submitted in this publication are based on our current knowledge and experience. They do not constitute a guarantee in the legal sense of the term and, in view of the manifold factors that may affect processing and application, do not relieve processors from the responsibility of carrying out their own tests and experiments. Any relevant patent rights and existing legislation and regulations must be observed.

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EXHIBIT 4